

What is Claimed is:

1. A purified and isolated catalytic domain from a  $\beta(1,4)$ -galactosyltransferase I that catalyzes formation of a galactose- $\beta(1,4)$ -N-acetylglucosamine bond in the presence of magnesium.
2. The catalytic domain according to claim 1, wherein the rate of formation of the galactose- $\beta(1,4)$ -N-acetylglucosamine bond is at least two-fold, five-fold, ten-fold, or one hundred-fold greater than wild-type  $\beta(1,4)$ -galactosyltransferase I in the presence of magnesium.
3. The catalytic domain according to claim 1, wherein the catalytic domain has a conservative amino acid exchange at an amino acid position corresponding to amino acid position 344 of SEQ ID NO: 6.
4. The catalytic domain according to claim 3, wherein histidine is exchanged for methionine at an amino acid position corresponding to amino acid position 344 of SEQ ID NO: 6.
5. The catalytic domain according to claim 1, further comprising a conservative amino acid substitution at an amino acid position corresponding to amino acid position 342 of SEQ ID NO: 6.
6. The catalytic domain according to claim 5, wherein threonine is exchanged for cysteine at amino acid position 342.
7. A polypeptide comprising the catalytic domain according to claim 1.
8. A purified and isolated catalytic domain from a  $\beta(1,4)$ -galactosyltransferase I that catalyzes formation of a glucose- $\beta(1,4)$ -N-acetylglucosamine bond in the presence of magnesium.
9. The catalytic domain according to claim 8, wherein the rate of formation of the glucose- $\beta(1,4)$ -N-acetylglucosamine bond is at least two-fold, five-fold,

ten-fold, or one hundred-fold greater than wild-type  $\beta(1,4)$ -galactosyltransferase I in the presence of magnesium.

10. The catalytic domain according to claim 8, wherein

(a) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 228 of SEQ ID NO: 6;

(b) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 229 of SEQ ID NO: 6; or

(c) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344, 228, and 229 of SEQ ID NO: 6.

11. The catalytic domain according to claim 10, wherein histidine is exchanged for methionine at amino acid position 344, and

(a) lysine is exchanged for arginine at amino acid position 228,

(b) glycine is exchanged for alanine at amino acid position 229, or

(c) lysine is exchanged for arginine at amino acid position 228, and glycine is exchanged for alanine at amino acid position 229.

12. The catalytic domain according to claim 8, further comprising a conservative amino acid substitution at an amino acid corresponding to amino acid position 342 of SEQ ID NO: 6.

13. The catalytic domain according to claim 12, wherein threonine is exchanged for cysteine at amino acid position 342.

14. A polypeptide comprising the catalytic domain according to claim 8.

15. A purified and isolated catalytic domain from a  $\beta(1,4)$ -galactosyltransferase I that catalyzes formation of an N-acetylgalactosamine- $\beta(1,4)$ -N-acetylglucosamine bond in the presence of magnesium.

16. The catalytic domain according to claim 15, wherein the catalytic domain has conservative exchanges of amino acids that correspond to amino acid positions 344 and 289 of SEQ ID NO: 6.
17. The catalytic domain according to claim 15, wherein histidine is exchanged for methionine at an amino acid position that corresponds to amino acid position 344 of SEQ ID NO: 6.
18. The catalytic domain according to claim 16, wherein tyrosine is exchanged for leucine, isoleucine, or asparagine at amino acid position 289.
19. The catalytic domain according to claim 15, further comprising a conservative amino acid substitution at an amino acid position that corresponds to amino acid position 342 of SEQ ID NO: 6.
20. The catalytic domain according to claim 19, wherein threonine is exchanged for cysteine at amino acid position 342.
21. A polypeptide comprising the catalytic domain according to claim 15.
22. A purified and isolated catalytic domain from  $\beta(1,4)$ -galactosyltransferase I that catalyzes formation of an N-acetylgalactosamine- $\beta(1,4)$ -glucose bond in the presence of  $\alpha$ -lactalbumin and magnesium.
23. The catalytic domain according to claim 22, wherein the catalytic domain has conservative amino acid exchanges at amino acid positions that correspond to amino acid positions 344 and 289 of SEQ ID NO: 6.
24. The catalytic domain according to claim 23, wherein histidine is exchanged for methionine at amino acid position 344.
25. The catalytic domain according to claim 23, wherein tyrosine at amino acid position 289 is exchanged with a leucine, isoleucine, or asparagine at amino acid position 289.

26. The catalytic domain according to claim 22, further comprising a conservative amino acid substitution at an amino acid position corresponding to amino acid position 342 in SEQ ID NO: 6.
27. The catalytic domain according to claim 26, wherein threonine is exchanged for cysteine at amino acid position 342.
28. A polypeptide comprising the catalytic domain according to claim 22.
29. A purified and isolated catalytic domain from a  $\beta(1,4)$ -galactosyltransferase I that catalyzes formation of an N-acetylglucosamine- $\beta(1,4)$ -N-acetylglucosamine bond in the presence of magnesium.
30. The catalytic domain of claim 29, wherein
  - (a) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 228 of SEQ ID NO: 6,
  - (b) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 289 of SEQ ID NO: 6, or
  - (c) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344, 228, and 289 of SEQ ID NO: 6.
31. The catalytic domain of claim 30, wherein
  - (a) lysine is exchanged for arginine at amino acid position 228,
  - (b) leucine is exchanged for tyrosine at amino acid position 289, or
  - (c) lysine is exchanged for arginine at amino acid position 228, and leucine is exchanged for tyrosine at amino acid position 289.
32. The catalytic domain according to claim 30, wherein histidine is exchanged for methionine at amino acid position 344.

33. The catalytic domain according to claim 29, further comprising a conservative amino acid substitution at an amino acid position corresponding to amino acid position 342 in SEQ ID NO: 6.
34. The catalytic domain according to claim 33, wherein threonine is exchanged for cysteine at amino acid position 342.
35. A polypeptide comprising the catalytic domain of claim 29.
36. A purified and isolated catalytic domain from a  $\beta(1,4)$ -galactosyltransferase I that catalyzes formation of a mannose- $\beta(1,4)$ -N-acetylglucosamine bond in the presence of magnesium.
37. The catalytic domain according to claim 36, wherein
  - (a) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 228 of SEQ ID NO: 6;
  - (b) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344 and 289 of SEQ ID NO: 6; or
  - (c) the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid positions 344, 228, and 289 of SEQ ID NO: 6.
38. The catalytic domain according to claim 37, wherein lysine is exchanged for arginine at amino acid position 228.
39. The catalytic domain according to claim 37, wherein leucine is exchanged for tyrosine at amino acid position 289.
40. The catalytic domain according to claim 36, further comprising a conservative amino acid substitution at an amino acid position corresponding to amino acid position 342 of SEQ ID NO: 6.

41. The catalytic domain according to claim 40, wherein threonine is exchanged for cysteine at amino acid position 342.
42. A polypeptide comprising the catalytic domain according to claim 36.
43. A purified and isolated catalytic domain from a  $\beta(1,4)$ -galactosyltransferase I that catalyzes formation of a galactose- $\beta(1,4)$ -N-acetylglucosamine-6-SO<sub>3</sub> bond in the presence of magnesium.
44. The catalytic domain according to claim 43, wherein the catalytic domain has conservative amino acid exchanges at amino acid positions corresponding to amino acid position 344, 279, and 280 of SEQ ID NO: 6.
45. The catalytic domain according to claim 44, wherein histidine is exchanged for methionine at an amino acid position that corresponds to amino acid position 344 of SEQ ID NO: 6.
46. The catalytic domain according to claim 44, wherein serine is exchanged for lysine at amino acid position 279.
47. The catalytic domain according to claim 44, wherein threonine is exchanged for phenylalanine at amino acid position 280.
48. A polypeptide comprising the catalytic domain according to claim 43.
49. A nucleic acid segment encoding a catalytic domain according to any one of claims 1, 8, 15, 22, 29, 36; or 43.
50. An expression cassette comprising the nucleic acid segment according to claim 49.
51. A cell comprising the nucleic acid segment according to claim 49, or the expression cassette according to claim 50.

52. A method to synthesize a galactose- $\beta$ (1,4)-N-acetylglucosamine moiety comprising incubating a reaction mixture comprising the catalytic domain according to claim 1, with a donor comprising galactose, and an acceptor comprising N-acetylglucosamine.

53. The method according to claim 52, wherein the donor is UDP-galactose and the acceptor is N-acetylglucosamine.

54. An oligosaccharide comprising a galactose- $\beta$ (1,4)-N-acetylglucosamine moiety synthesized according to the method of claim 52.

55. A method to synthesize a glucose- $\beta$ (1,4)-N-acetylglucosamine moiety comprising:

incubating a reaction mixture comprising the catalytic domain according to claim 8, with a donor comprising glucose, and an acceptor comprising N-acetylglucosamine.

56. The method according to claim 55, wherein the donor is UDP-glucose, the acceptor is N-acetylglucosamine, or the donor is UDP-glucose and the acceptor is N-acetylglucosamine.

57. An oligosaccharide comprising a glucose- $\beta$ (1,4)-N-acetylglucosamine moiety synthesized according to the method of claim 55.

58. A method to synthesize an N-acetylgalactosamine- $\beta$ (1,4)-N-acetylglucosamine moiety comprising:

incubating a reaction mixture comprising the catalytic domain according to claim 15, with a donor comprising N-acetylgalactosamine, and an acceptor comprising N-acetylglucosamine.

59. The method according to claim 58, wherein the donor is UDP-N-acetylgalactosamine, the acceptor is N-acetylglucosamine, or the donor is UDP-N-acetylgalactosamine and the acceptor is N-acetylglucosamine.

60. An oligosaccharide comprising an N-acetylgalactosamine- $\beta$ (1,4)-N-acetylglucosamine moiety synthesized according to the method of claim 58.
61. A method to synthesize an N-acetylgalactosamine- $\beta$ (1,4)-glucose moiety comprising incubating a reaction mixture comprising the catalytic domain according to claim 22,  $\alpha$ -lactalbumin, a donor comprising N-acetylgalactosamine, and an acceptor comprising glucose.
62. The method according to claim 61, wherein the donor is UDP-N-acetylgalactosamine, the acceptor is glucose, or the donor is UDP-N-acetylgalactosamine and the acceptor is glucose.
63. An oligosaccharide comprising an N-acetylgalactosamine- $\beta$ (1,4)-glucose moiety synthesized according to the method of claim 61.
64. A method to synthesize an N-acetylglucosamine- $\beta$ (1,4)-N-acetylglucosamine moiety comprising incubating a reaction mixture comprising a catalytic domain according to claim 29, with a donor comprising N-acetylglucosamine, and an acceptor comprising N-acetylglucosamine.
65. The method according to claim 64, wherein the donor is UDP-N-acetylglucosamine, the acceptor is N-acetylglucosamine, or the donor is UDP-N-acetylglucosamine and the acceptor is N-acetylglucosamine.
66. An oligosaccharide comprising an N-acetylglucosamine- $\beta$ (1,4)-N-acetylglucosamine moiety synthesized according to the method of claim 64.
67. A method to synthesize a mannose- $\beta$ (1,4)-N-acetylglucosamine moiety comprising incubating a reaction mixture comprising the catalytic domain according to claim 36, with a donor comprising mannose, and an acceptor comprising N-acetylglucosamine.

68. The method according to claim 67, wherein the donor is UDP-mannose, the acceptor is N-acetylglucosamine, or the donor is UDP-mannose and the acceptor is N-acetylglucosamine.

69. An oligosaccharide comprising a mannose- $\beta(1,4)$ -N-acetylglucosamine moiety synthesized according to the method of claim 67.

70. A method to synthesize a galactose- $\beta(1,4)$ -N-acetylglucosamine-6-SO<sub>3</sub> moiety comprising incubating a reaction mixture comprising the catalytic domain according to claim 43, with a donor comprising galactose, and an acceptor comprising N-acetylglucosamine-6-SO<sub>3</sub>.

71. The method according to claim 70, wherein the donor is UDP-galactose, the acceptor is N-acetylglucosamine-6-SO<sub>3</sub>, or the donor is UDP-galactose and the acceptor is N-acetylglucosamine-6-SO<sub>3</sub>.

72. An oligosaccharide comprising a galactose- $\beta(1,4)$ -N-acetylglucosamine-6-SO<sub>3</sub> moiety synthesized according to the method of claim 70.

73. A method comprising incubating a reaction mixture comprising an antigen having an acceptor, a donor, and the catalytic domain according to any one of claims 1, 8, 15, 22, 29, 36, or 43 under conditions wherein the  $\beta(1,4)$ -galactosyltransferase I catalyzes bond formation between the donor and the acceptor on the antigen and causes an increase in the immunogenicity of the antigen.

74. The method according to claim 73, wherein the donor is selected from the group consisting of UDP-galactose, UDP-mannose, UDP-N-acetylglucosamine, UDP-glucose, GDP-mannose, and UDP-N-acetylgalactosamine.

75. The method according to claim 73, wherein the acceptor is a carbohydrate, a glycoprotein, or a glycolipid.

76. The method according to claim 75, wherein the carbohydrate is selected from the group consisting of a monosaccharide, a disaccharide, an oligosaccharide, and a polysaccharide.

77. The method according to claim 73, wherein the antigen is a vaccine.

78. The method according to claim 73, wherein the antigen is a protein or a glycoprotein.

79. An antigen prepared according to the method of claim 73.

80. A method to prepare a saccharide composition having a defined sequence comprising:

contacting an acceptor with a first donor in the presence of a first catalytic domain to catalyze linkage of the acceptor with the donor to form a first saccharide composition; and

contacting the first saccharide composition with a second donor in the presence of a second catalytic domain to catalyze linkage of the first saccharide composition with the second donor to form a second saccharide composition,

wherein at least the first catalytic domain or the second catalytic domain is a catalytic domain according to any one of claims 1, 8, 15, 22, 29, 36, or 43, and the other first or second glycosyltransferase is selected from the group consisting of a galactosyltransferase, a sialyltransferase, a fucosyltransferase, an N-acetylgalactosaminyltransferase, an N-acetylglucosaminyltransferase, and a glucuronyltransferase.

81. The method according to claim 80, wherein the first donor or the second donor is selected from the group consisting of UDP-galactose, UDP-mannose, UDP-N-acetylglucosamine, UDP-glucose, GDP-mannose, UDP-N-acetylgalactosamine, UDP-glucuronic acid, GDP-Fucose, and CMP-N-acetylneurameric acid.

82. The method according to claim 80, wherein the acceptor is a carbohydrate, a glycoprotein, or a glycolipid.

83. The method according to claim 82, wherein the carbohydrate is selected from the group consisting of a monosaccharide, a disaccharide, an oligosaccharide, and a polysaccharide.

84. The method according to claim 80, wherein the acceptor is an antigen.

85. The method according to claim 84, wherein the antigen is a vaccine.

86. The method according to claim 84, wherein the antigen is a protein or a glycoprotein.

87. A composition prepared according to the method of claim 80.

88. A kit comprising packaging material, and a polypeptide comprising the catalytic domain of any one of claims 1, 8, 15, 22, 29, 36, or 43.

89. The kit according to claim 88, further comprising a donor.

90. The kit according to claim 89, wherein the donor is selected from the group consisting of UDP-galactose, UDP-mannose, UDP-N-acetylglucosamine, UDP-glucose, GDP-mannose, UDP-N-acetylgalactosamine, UDP-glucuronic acid, GDP-Fucose, and CMP-N-acetylnneuraminiic acid.

91. A method to link a donor into an acceptor that is attached to a blood platelet comprising

contacting the blood platelet with a donor and at least one catalytic domain according to any one of claims 1, 8, 15, 22, 29, 36, or 43 to form a reaction mixture, and  
incubating the reaction mixture under conditions where the catalytic domain catalyzes linkage of the donor to the acceptor.

92. The method according to claim 91 wherein the donor is exogenous UDP-galactose.

93. The method according to claim 91, wherein the donor is selected from the group consisting of UDP-galactose, UDP-mannose, UDP-N-acetylglucosamine, UDP-glucose, GDP-mannose, UDP-N-acetylgalactosamine, UDP-glucuronic acid, GDP-Fucose, and CMP-N-acetylneuraminic acid.

94. The method according to claim 91, wherein the acceptor is a carbohydrate, a glycoprotein, or a glycolipid.

95. The method according to claim 91, wherein the acceptor is N-acetylglucosamine.